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SGM 6934.1
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Ward et al.
Serial No.: 09/610,935
Filed: July 6, 2000
For: TARGET REAGENTS THAT ENHANCE REACTION-PRODUCT ANALYSIS
Examiner: Bradley L. Sisson
Confirmation No. 5148

Art Unit: 1655

August 1, 2001

RESPONSE UNDER 37 C.F.R. 1.111

TO THE COMMISSIONER OF PATENTS AND TRADEMARKS,

Sir:

In response to the Office action issued on April 4, 2001, the due date for which having been extended one (1) month to August 4, 2001, Applicants respectfully submit the following amendments and remarks in connection with the above-identified application.

Amendment A

In the Specification:

The paragraph beginning at line 1 of page 29 has been amended as follows:

A preferred composition was formulated at 1 u/µl Taq polymerase in Taq storage buffer (consisting of 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% TWEEN 20 (Polyoxyethylenesorbitan monolaurate), 0.5% Igepal® CA-630, 50% glycerol in water) with the magnesium formulation of dye at a total absorbance of 300. The dye composition was 80% acid red 1, 20% acid violet 5 (100%= absorbance of acid red 1 at λ_{max} + absorbance of acid violet 5 at λ_{max}, absorbance of acid red 1=240, acid violet 5=60). This formulation is designated "*REDTaq*™". When added to a PCR reaction mixture at 0.05 u/µl Taq, the total dye absorbance is 15. The dye combination at this concentration was visible in a subsequent agarose gel electrophoresis of the completed reaction mix, yet the combination was relatively non-toxic to PCR. A lower concentration of the dye in the reaction mixture would be difficult to see during a subsequent agarose gel electrophoresis. As a comparison, the previously

discussed prior art Taq-dye formulation, Red Hot DNA Polymerase, has an absorbance of 3.3 at 572 nm, and 4.6 at 435 nm. At the recommended concentration in a PCR reaction mixture, Red Hot DNA Polymerase has an absorbance of 0.033 and 0.046, at 572 and 435 nm, respectively. Therefore, in contrast to *REDTaq*™, the Red Hot DNA Polymerase formulation would not be useful as a tracer in an electrophoretic analysis of a PCR reaction.

Q1

In the claims:

Claim 12 has been amended as follows:

12.(amended) The composition of claim 11 wherein the reaction component is essential for an ex-vivo polymerase reaction in which a nucleic acid polymer product complementary to a nucleic acid polymer template is prepared, the tracer is compatible with the polymerase, and the composition is substantially free of the nucleic acid polymer template.

Q2

Please add new claims 34 through 41 as follows:

34. (new) The composition of claim 1 wherein the reaction component is essential for an ex-vivo enzymatic reaction in which a nucleic acid polymer substrate is enzymatically cleaved by a restriction enzyme in a reaction mixture to form a restriction product, the tracer compatible with the restriction enzyme, and the composition is substantially free of the nucleic acid polymer substrate.

35. (new) The composition of claim 34 wherein the density of the composition is at least about 1.01 g/cm^3.

Q3

36. (new) The composition of claim 34 wherein the density of the composition is at least about 1.1 g/cm^3.

37. (new) The composition of claim 34 wherein the optical density of the composition is at least about 15 at a visible wavelength of maximal tracer absorbance.

38. (new) The composition of claim 34 wherein the optical density of the composition is about 200 - 400 at a visible wavelength of maximal tracer absorbance.

39. (new) The composition of claim 34 wherein the reaction component essential for a polymerase reaction is a concentrated buffer solution.

Q13.

40. (new) The composition of claim 34 wherein the reaction component essential for an enzymatic reaction comprises a restriction endonuclease.

41. (new) The composition of claim 34 wherein the tracer is amaranth.

REMARKS

With this amendment, claims 1-22 and 34-41 are pending. Reconsideration of the present application are respectfully requested in view of the present amendments and remarks.

Restriction Requirement

The Office has required restriction under 35 U.S.C 121 to either Group I, claims 1-22, which are drawn to compositions suitable for formulation of an enzymatic reaction mixture; Group II, claims 23-27, which are drawn to methods for a polymerase reaction; or group III, claims 28-33, which are drawn to methods for a restriction enzyme reaction. On March 28, 2001, Applicants provisionally elected Group I, with traverse. Applicants hereby affirm the election of the claims of Group I.

However, Applicants respectfully request examination of the Group II and III claims with the elected Group I claims. Although the inventions defined by the claims of Group I are distinct or independent from those of Groups II and III, the search and examination of these claims can be made without additional burden on the Examiner. In such instances, the Examiner must examine them on the merits.[1]

Group I claims (claims 1-22) are directed to compositions comprising a reaction component essential for an *ex vivo* non-polymerase enzymatic reaction in which a substrate is catalyzed by an enzyme in a reaction mixture to form a product and a tracer compatible with the enzyme wherein the composition is substantially free of the substrate. These compositions are intimately related to the claims of Group II (claims 23-27) which are directed to methods for a polymerase reaction and the claims of Group III (claims 28-33) which are directed to methods for a restriction enzyme reaction. Specifically, the compositions of the claims of Group I are included in the steps of the method claims of Group II and Group II.

Hence, in view of the relationship in several significant aspects of the claims of Groups I, II and III, the searches required will not present an undue burden to the Examiner. Accordingly, rejoinder of the Group II and II claims is proper and is respectfully requested.

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[1] *See* MPEP § 803.

In the event that the Examiner does not allow rejoinder of Groups II and III, Applicants reserve the right to file divisional applications directed to the non-elected claims of these groups.

Amendments to the Specification

As suggested by the Examiner, the specification has been amended at page 29, line 4 to provide the chemical name for TWEEN 20. No new matter has been added.

Amendments to the Claims

Claim 12 has been amended to provide antecedent basis.

New claims 34-41 have been added. Support for claims 34-41 can be found in the specification at page 7, lines 1-8 and Example 2.

No new matter has been added by these claim amendments or new claims.

Rejections under 35 U.S.C. § 112

Written Description

Reconsideration and withdrawal are requested of the rejection of claims 1-22 under 35 U.S.C. § 112, first paragraph. The Office asserts that it is not clear that Applicants were in possession of the invention as claimed in claims 1-22 because the working examples disclose only one enzyme (*i.e.*, Taq enzyme) and the specification does not identify which tracers would be suitable for use with the enzymes listed in the specification.[2] For the reasons set out herein, Applicants respectfully traverse.

Claim 1 is directed to a composition suitable for formulation of an enzymatic reaction mixture comprising a reaction component essential for an *ex-vivo* non-polymerase enzymatic reaction in which a substrate is catalyzed by an enzyme, and a tracer compatible with the enzyme, wherein the composition is substantially free of the substrate. Claim 11 is directed to a composition comprising a reaction component essential for an *ex-vivo* enzymatic reaction in which a substrate is catalyzed by an enzyme, and a tracer compatible with the enzyme, wherein

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[2] *See* Office action at page 5.

the composition is substantially free of the substrate and has an optical density greater than about 5 at a visible wavelength of maximal tracer absorbance.

The specification discloses several types of enzymes which modify or degrade proteins, lipids, carbohydrates, and metabolites, such as kinases, proteases, lipases, amylases, peroxidases, oxidases, oxygenases, and dehydrogenases as well as enzymes which modify, cut, or synthesize nucleic acids.[3] In particular, the specification discloses several nucleic acid modifying enzymes including DNA polymerases, particularly thermostable DNA polymerases such as wild-type or modified Taq polymerase, RNA polymerases, ligases, kinases, restriction endonucleases, phosphodiesterases, DNases, exonucleases, RNases, and phosphatases which are typically utilized in laboratory enzymatic transformations.[4] Further, the specification discloses several examples of polymerase and non-polymerase reactions in which a substrate is catalyzed by an enzyme such as the polymerase chain reaction ("PCR"), sequencing, southern hybridization analysis, restriction endonuclease analysis, RNase protection, and the production of labeled probes.[5] With regard to the tracer, the specification provides a list of tracers in Table 1[6] and discloses that the tracer is compatible with the enzyme and is preferably anionic.[7] Example 1 describes the methods and selection criteria utilized by the Applicants to select tracers which are compatible with the enzyme for use in a polymerase chain reaction. Example 2 describes the procedure utilized by the Applicants to determine the compatibility of a dye with a restriction endonuclease. The restriction enzymes were relatively insensitive to dye addition and as such, the criteria for determining the compatibility of the tracer with a restriction enzyme in a composition (claim 1) is the same as the criteria disclosed in Example 1 for compositions for use in polymerase reactions (claim 11).[8] Specifically, Example 1 describes the following

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[3] *See* Specification at page 1, lines 13-20; page 11, line 30 to page 12, line 20.

[4] *Id.*

[5] *See* Specification at page 1, lines 22-25.

[6] *See* Table 1, pages 19-23 of the Specification.

[7] *See* Specification at page 12, line 21 to page 12, line 35.

[8] *See* Specification at page 25, lines 31, lines 9-19; Figure 17.

characteristics of a dye which indicate compatibility with an enzyme: color; solubility; ethanol precipitation; PCR toxicity; ligase toxicity; transformation toxicity; and reverse phase desalting.[9] The Patent Office has recognized that the disclosure of relevant identifying characteristics of a representative number of species may satisfy the written description requirement with respect to the genus[10] and as demonstrated above, the specification clearly describes the tracer in the composition of claim 1 and in claim 11 in terms of its physical, chemical and functional characteristics. Accordingly, Applicants submit that one skilled in the art would recognize that the inventors were in possession of the composition of claim 1 and the composition of claim 11 and request that this basis for rejection be withdrawn.

Claims 2-10 are dependent upon claim 1 and claims 12-22 are dependent on claim 11 and are adequately described for the same reasons set out above.

Enablement

The Office has rejected claims 1-22 as containing subject matter which is not described in the specification in such a way to enable one skilled in the art to practice the invention.[11] Applicants traverse and respectfully submit that the compositions of claims 1-22 are fully enabled when the description, specific examples and the high level of knowledge in the art are considered.

The Office asserts that the claims are broad in scope and encompass compositions comprised of any of a large number of enzymes with a dye.[12] As discussed above with regard to written description, the specification discloses several types of enzymes typically utilized in laboratory transformations[13] and examples of polymerase and non-polymerase reactions in which

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[9] *See* Specification at page 25, line 1 to page 28, line 15; Figure 5.

[10] *See* "Revised Interim Guidelines for Examination of Patent Application Under 35 U.S.C. Sec. 112 'Written Description' Requirement; Request for Comments", 64 Fed. Reg. 71427, 71436 (1999).

[11] *See* Office action at page 7.

[12] *See* Office action at pages 8-9.

[13] *See* Specification at page 1, lines 13-20; page 11, line 30 to page 12, line 20.

a substrate is catalyzed by an enzyme.[14] A patent need not teach and preferably omits, what is well known in the art[15] and Applicants submit that polymerase and non-polymerase enzymatic reactions are commonly conducted by those skilled in the art and as such, a skilled artisan would know which enzymes to utilize in such reactions.

The Office further states that other than performing PCR with Taq polymerase, the specification does not disclose reaction conditions employed for using the composition of claim 1 or 11.[16] The composition of claim 1 and claim 11 are utilized in enzymatic reactions which are commonly performed in the art and as such, a skilled artisan would know the conditions for conducting reactions such as a restriction enzyme reaction or PCR. Indeed, DNA digestion using restriction enzymes is commonly performed in molecular biology and the polymerase chain reaction is described as a "**widely used** technique in molecular biology."[17] Conditions for PCR are well known in the art and can be found, for example in Chapter 1 of Innis et al., *PCR Protocols*, Academic Press, 1990.

The specification contains working examples which clearly describe the methods used to prepare the compositions and as such, the specification is enabling for a composition comprising a reaction component and a tracer as claimed in claim 1 and as in claim 11. In particular, Example 1 provides guidance as to the methods utilized by the Applicants to make a formulation containing Taq polymerase as the essential reaction component for a enzymatic reaction, and a red dye as the tracer which is compatible with the Taq polymerase. The steps taken to develop this composition are detailed in Example 1 and summarized in Figure 5. Specifically, guidance as to the methods utilized to evaluate the physical and chemical characteristics such as color, solubility, ethanol precipitation and PCR toxicity[18] of the red dye is provided which would enable a skilled artisan to make compositions containing an enzyme and a tracer compatible with

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[14] *See* specification at page 1, lines 22-25.

[15] *See In re Buchner*, 18 U.S.P.Q.2d 1331, 1332 (Fed. Cir. 1991).

[16] *See* Office action at page 9.

[17] *The Encyclopedia of Molecular Biology*, Blackwell Science, 1994, p. 864 (emphasis added).

[18] *See* Specification at page 25, line 1 to page 27, line 12; Table 2.

the enzyme *see e.g.*, the specific steps required to conduct the PCR toxicity study are disclosed in Example 1.[19] Likewise, Example 2 discloses the methods and criteria utilized by the Applicants in determining the compatibility of a dye with a restriction enzyme to produce a composition including a restriction enzyme as the reaction component essential for an *ex-vivo* non-polymerase enzymatic reaction, and amaranth as the tracer compatible with the restriction enzyme.

Thus, the specification provides guidance as to how to screen tracers to determine if the tracer possesses the characteristics indicating compatibility with the enzyme. Such screening methods utilized to identify tracers compatible with an enzyme are well-known techniques of molecular biology. Accordingly, if screening is necessary to make the compositions as described, such screening does not support a rejection under §112, first paragraph because the screening is considered to be routine and the specification provides guidance with respect to the direction in which the experimentation should proceed.[20] Guidance as to the methods and criteria used to screen a tracer to determine its compatibility with an enzyme and to produce a composition of claim 1 or claim 11 is given in the specification and specifically in Examples 1-2.

This guidance must be taken as enabling unless the Patent Office can provide "acceptable evidence or reasoning which is inconsistent with the contested statement."[21] However, the Office presents no objective evidence of lack of enablement, only conclusory statements. In this regard, Applicants note *Ex parte Cook*[22] and *In re Kamal*[23] which provide that a rejection based on an allegation of inoperativeness of members of a claimed class of compounds must be supported either by tangible reasons in support of the doubt or evidence. The USPTO may not judicially notice the "inoperability" of species of compounds encompassed within a claim.[24] Also, M.P.E.P. § 2164.05 admonishes Examiners never to make a determination as to whether a

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[19] *See* specification at page 25, line 22 to page 26, line 19.

[20] *In re Wands* 858 F.2d, 731, 737 (Fed. Cir. 1988).

[21] *See In re Marzocchi*, 439 F.2d, 220, 224, 169 U.S.P.Q. 367, 370 (C.C.P.A. 1971).

[22] 105 U.S.P.Q. 504 (B.P.A.I. 1955).

[23] 158 U.S.P.Q. 320 (C.C.P.A. 1968).

[24] *In re Kamal* at 323.

claimed invention is enabled based on personal opinion. Specific technical reasons are always required.

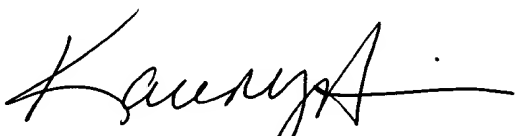
Thus, the rejection for lack of enablement of claims 1-22 is improper and Applicants respectfully request that it be withdrawn.

CONCLUSION

Reconsideration of the present application are respectfully requested in view of the present amendments and remarks. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version With Markings To Show Changes Made"**.

In light of the above amendments and remarks, Applicants respectfully requests favorable reconsideration of the present application. Enclosed is a check for $110.00 to cover the fees for the one (1) month extension of time. Any fee deficiency may be charged to Deposit Account No. 19-1345.

Respectfully submitted,



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